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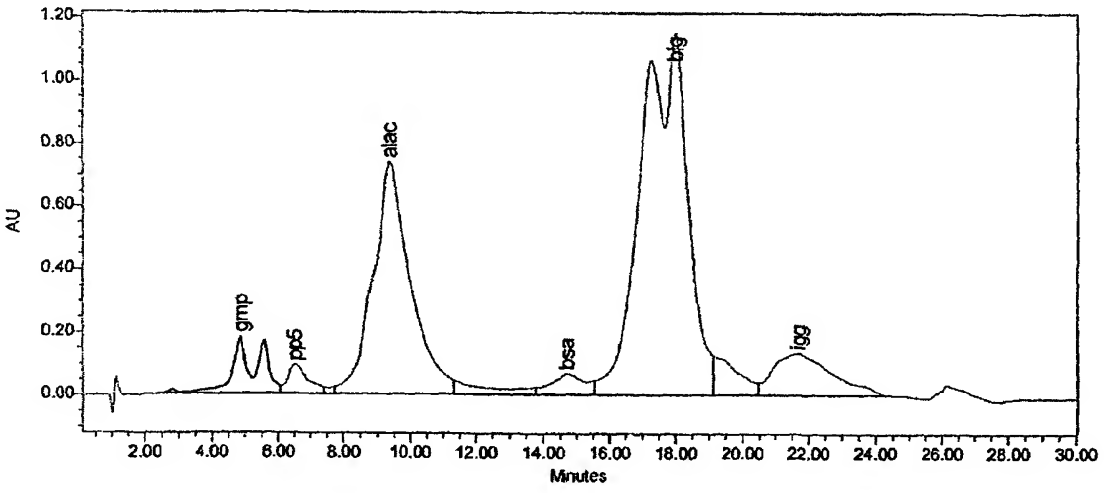
(54) Title: METHOD OF OBTAINING GLYCO-MACRO-PEPTIDE DEPLETE PRODUCT FROM WHEY



(57) Abstract: The invention involves a whey protein product derived from sweet whey by anion exchange which is acid heat stable and/or deplete in glyco-macro-peptide (GMP), and methods of producing such a product. The method involves taking a sweet whey stream, acidified the stream to a pH in the range substantially 4 to 6, subjecting the acidified stream to anion exchange and collecting the non-bound protein stream. The preferred product has absorbence at 610nm of less than or equal to 0.100 AU/cm after it has been heated at 60-80 °C, pH 3.6-3.8 for 20 minutes, and with a protein concentration of 4.8-5 %. The preferred product also has a GMP content that is less than the content of the starting whey stream, and preferably no more than 15 % of the total protein content. More preferably the GMP content may be no more than 3 % of the total protein content. The product has particular application as protein fortification for low pH beverages.

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METHOD OF OBTAINING GLYOC-MACROPEPTIDE DEPLETE PRODUCT FROM WHEY

FIELD OF THE INVENTION

This invention relates to a method of obtaining a whey product deplete of Glyco-Macro-Peptide (GMP) which is heat stable at low pH from a sweet whey, through the use of anion exchange, and products obtained from the method.

BACKGROUND TO THE INVENTION

GMP describes a set of long-chain amino acids which occur during the manufacture of cheese and of rennet casein. GMP is the product of the hydrolysis of Kappa-casein by a suitable rennet enzyme. GMP therefore occurs in all cheese whey and rennet casein whey.

Many processes exist for the recovery of GMP from sweet whey (e.g. US 5,916,621, US 5,061,622, US 5,968,586, US 5,075,424, US 5,216,129, US 5,278,288, US 5,280,107, WO 98/14071 and GB 2188526). These processes are generally concerned with the recovery of the GMP fraction of whey, which comprises approximately 20% of the protein content of sweet whey.

No process has focussed on a GMP-deplete whey protein stream. Furthermore, it has generally been considered that GMP contributes to acid heat stability, and that, therefore, GMP-deplete products would not be useful in low pH conditions where heat stability was required.

In this specification, the term acid heat stable refers to a product in which the turbidity is quantified by measuring the absorbance at

610nm and is found to be less than 0.100 AU cm^{-1} after it has been heated at 60-80°C, pH 3.6-3.8, for 20 minutes. The protein concentration in the solution for the purposes of this test is 4.8-5%.

Alternatively the protein concentration may be equal to, or greater than that found in whey (0.8%) in which case an absorbance at 610nm of less than 0.030 AU cm^{-1} after 20 minutes at 80°C, proves acid heat stability.

The term GMP-deplete whey product refers to a sweet whey stream that has had a degree of GMP removal performed on it via anion exchange.

It is an object of the present invention to provide an acid heat stable, GMP-deplete whey product and/or a method of its production which reduces or overcomes the above mentioned problems, or at least provides the public with a useful alternative.

Other objects of the invention may become apparent from the following description, which is given by way of example only.

SUMMARY OF THE INVENTION

According to one aspect of the invention there is provided a whey protein product derived from sweet whey by ion exchange using anion medium exchange which is acid heat stable.

Preferably, the whey protein is GMP-deplete.

Preferably, the product may include no more than 15% GMP as a proportion of the total protein.

Preferably the acid heat stability will be such that at pH 3.6 the application of heat will not result in any observable lack of clarity.

Preferably the acid heat stability may be such that the absorbance at 610nm at a pH of substantially 3.6 and heating to a temperature of substantially 80 °C for substantially 20 minutes, of a solution containing substantially 5% protein, is ≤ 0.030 AU/cm.

Preferably, the absorbance under those conditions may be ≤ 0.01 AU/cm.

According to a further aspect of the present invention, there is provided a method of producing a whey protein product which is acid heat stable, from sweet whey, including the steps of:

- taking a sweet whey stream;
- acidification of the stream to a pH in the range substantially 4 to 6;
- subjecting the acidified stream to ion exchange with anion exchange medium;
- collecting the non bound protein stream.

Preferably, the method may further include ultrafiltration of the non-bound protein stream.

Preferably, the breakthrough stream may be ultrafiltered and diafiltered with water to produce a retentate having a total solids content of 18-28% total solids with a protein content of 90% or greater on a dry basis.

Other aspects of the invention may become apparent from the following description, which is given by way of example only.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Chromatogram of product produced by Example 2.

DETAILED DESCRIPTION OF THE INVENTION

In broad terms, the method of the invention begins by taking a whey or whey retentate, acidification of the whey stream to a pH in the range 4 to 6, and then subjecting the acidified whey stream to anion exchange to produce a stream deplete in GMP.

The feed whey material may be a cheese whey, rennet whey, cheese whey protein concentrate, rennet whey protein concentrate, cheese whey protein isolate or rennet whey protein isolate.

The whey stream may be subject to a process such as microfiltration to remove particulate material which may be present and may cause blocking of a packed ion exchange bed.

The acidified whey stream may then be applied to a packed bed column including an anion exchange resin such as SepraPrep Q, which has been previously regenerated. The regeneration may be with mineral or organic acids, salt or a mixture of salts (such as sodium chloride, potassium chloride, or calcium chloride) or a mixture of acid(s) and salt(s). The breakthrough stream from the anion exchange process may be ultrafiltered and diafiltered with water to produce a retentate, which may then be spray dried.

It will be appreciated that alternatives to packed bed ion exchange, such as expanded bed systems, fluidised bed systems or stirred tank systems, may be used. Radial flow columns may be employed as opposed to conventional axial flow columns.

Furthermore, alternative salts such as sodium chloride, potassium chloride, or calcium chloride may be used instead of acid to regenerate the ion exchange bed.

The product may be processed and dried at pH 3.4-5.0, or it may be neutralised and dried.

The end product has a GMP level, less than that of the original whey stream. Preferably, the GMP content is less than 15% of the total protein content, and it may be below 2% of the total protein content. Surprisingly, the product is acid heat stable.

EXAMPLE 1

3000 litres of rennet whey protein concentrate retentate (17% total solids, 9.5% protein) was diluted with water until the protein content was 1.5%. The diluted retentate was microfiltered. The microfiltered permeate was adjusted to pH 4.7 with 98% sulphuric acid.

500 litres of pH 4.7 MF permeate was applied to a 50 litre ion exchange column (bed height of 10cm) packed with SepraPrep Q anion exchange resin which had previously been regenerated with 1M Hydrochloric Acid (1M HCl). The non bound protein stream was collected and ultrafiltered using Koch HFK 328 membranes (5 kD MWCO) and diafiltered with water to produce a retentate of 18% total solids (90% protein dry basis). The retentate was then spray dried.

The resulting powder was found to have exceptional heat stability. When 5% protein solutions were made up and pH adjusted (either with citric acid or with sodium hydroxide, as appropriate), absorbance at 610nm was measured both before and after heating at 60°C for 20 minutes. The results are shown in Table I.

Table I:

Absorbance at 610nm (5% protein solution)	Unheated	Heated
Natural pH (3.74)	Nil	0.013
pH 3.8	0.007	0.006

These absorbance levels show that the protein solution is clear, and remains clear after heating.

Flavour was tested in a solution of 5% protein with 6% fructose, and compared to other commercially available whey Protein Isolates. 8 out of 10 untrained panellists regarded the flavour of the current invention as more desirable than the other solutions.

EXAMPLE 2

The process of Example I was repeated to produce the retentate of 18% total solids from the breakthrough stream. The retentate was pH adjusted to near pH 6.8 with a mixture of 2.5% sodium hydroxide and 2.5% potassium hydroxide, before being spray dried.

This powder was tested for its functional characteristics of solubility, and acid stability, as shown in Tables II, and III.

20g of powder were reconstituted in distilled water, made up to 400g. Whilst the solution was stirred, NaOH or HCl was added to increase or decrease the pH. Samples were withdrawn at specified pH values (±0.01), centrifuged and the volume of sediment measured. The results are shown in Table II.

TABLE II:

Solubility Profile – Sediment (ml/10ml of solution, solution =5%TS)

pH	Natural (pH 6.90)	2.0	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	8.0
Sediment	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

Thus, no precipitation occurs over a wide range of pH conditions.

TABLE III:

Acid Stability

This was measured using the Acid Beverage Rapid Test. The powder was reconstituted at 3% protein concentration, adjusted to pH 3.8 with phosphoric acid (or sodium hydroxide), heated to 80°C for 20 minutes, cooled and allowed to stand for 6 hours.

Absorbance at 610nm for a sample at 3% protein, pH 6.93, was 0.010. Absorbance was then measured at various pH levels, before and after heating at 80°C for 20 minutes, and after sitting overnight.

Final pH	3.99		3.80		3.50	
Treatment	Unheated	Heated	Unheated	Heated	Unheated	Heated
Appearance after heating	Very Clear	Slightly Murkier	Very Clear	Very Clear	Very Clear	Very Clear
Sediment after sitting overnight	0 ml / 15ml solution	0 ml / 15ml solution	0 ml / 15ml solution	0 ml / 15ml solution	0 ml / 15ml solution	0 ml / 15ml solution
Absorbance after sitting overnight	0.013	0.118	0.007	0.024	0.012	0.005

These results demonstrate that the solution remains clear at reduced pH levels after heating. Generally, turbidity was ≤ 0.02 absorbance units.

GMP Content

GMP content was measured by reverse-phase HPLC (column - Pharmacia Resource RPC 1ml flow rate 1 ml/min; injection volume 20 μl; start buffer 0.1% trifluoroacetic acid in water; end buffer: 90% acetonitrile, 0.1% trifluoroacetic acid in water; UV detection at 214 nm). The results are shown in Figure 1, which identifies the individual peaks. The level of GMP was 4.8% of protein.

EXAMPLE 3

Rennet whey protein concentrate retentate was diluted to 1.9% protein and microfiltered. A 50 ml bed (10cm bed depth) of Pharmacia Q Sepharose Big Beads was regenerated with 1M HCl then washed with demineralised water. The microfiltered whey stream was adjusted to pH 4.43 with sulphuric acid and passed through the anion exchange resin at 3m/hr. Samples of the non bound protein fraction were collected during the run and tested for GMP content and acid stability.

Sample	GMP (% of total protein)	610nm abs (post 80°C, pH 3.6)
Column Feed	17.1%	0.953
Non bound stream 0-5 column volumes	1.11%	0.007
5-6 column volumes	1.21%	0.005
6-7 column volumes	1.21%	0.006
7-8 column volumes	1.37%	0.006
8-9 column volumes	1.54%	0.006
9-10 column volumes	2.57%	0.004
10-12 column volumes	13.91%	0.008

As seen from the results above a significant improvement in acid stability is achieved and the GMP content of the whey material is reduced.

EXAMPLE 4

Whey material was prepared for ion exchange by microfiltering rennet whey protein concentrate diluted to 1.9% protein. A 50 ml bed (10cm bed height) of anion exchange resin, Sepraprep Q, was regenerated with 1M HCl. The microfiltered feed material was split into 4 batches and the batches were adjusted to pHs 5.25, 5.00, 4.75, and 4.55 using sulphuric acid. The batches were then passed through the anion exchange resin at 3m/hr, with 1M HCl followed by a water flush being used to regenerate the column between batches. Samples of the non bound protein fraction were collected during the run and tested for GMP content and acid stability.

Sample	GMP (% of total protein)	610nm abs (post 80°C, pH 3.6)
Column Feed	18.5%	0.239*
Non bound stream **pH 5.25** 0-4.5 column volumes	2.99%	0.004
pH 5.25 4.5-6 column volumes	2.23%	0.005
pH 5.25 6-10 column volumes	3.36%	0.004
Non bound stream **pH 5.00** 0-4.5 column volumes	6.20%	0.007
pH 5.00 4.5-6 column volumes	3.08%	0.004
pH 5.00 6-10 column volumes	3.89%	0.004
Non bound stream **pH 4.75** 0-4.5 column volumes	5.17%	0.008
pH 4.75 4.5-6 column volumes	5.42%	0.008
pH 4.75 6-10 column volumes	9.12%	0.006
Non bound stream **pH 4.55** 0-4.5 column volumes	9.29%	0.008
pH 4.55 4.5-6 column volumes	13.11%	0.007
pH 4.55 6-10 column volumes	17.16%	0.010

* The lower than expected absorbance measurement for the column feed material is explained by the formation of discrete aggregates in this sample which gives fluctuating absorbance measurements.

EXAMPLE 5

Rennet whey protein concentrate was diluted to 1.9% protein and microfiltered to prepare it for ion exchange. A 50L ion exchange column (10cm bed height) was packed with Sepraprep Q anion exchange resin and regenerated with 1 M HCl then washed with demineralised water. The microfiltered whey was adjusted to pH 5.25 with sulphuric acid and 800L was passed through the resin. The resin was then regenerated again with 1 M HCl and the process repeated. The non bound protein stream from both cycles was then ultrafiltered and diafiltered at pH 3.6 using 5kD ultrafiltration membranes (Koch HFK328s). A retentate of 16.5 % total solids (90% protein on a dry basis) was produced.
A sample of this retentate was diluted to 5% protein and subjected to 80°C for 20 minutes. Absorbance at 610nm was measured at 0.007 AU cm^{-1}. The heated sample appeared completely clear at pH 3.6.

COMPARATIVE ANALYSIS

The acid heat stability of the GMP-deplete product of the invention may be compared to that of a whey protein product produced by cation exchange, having a GMP level in the range 5% of total protein, which failed to have the functional characteristic of clarity and stability in acid and heat-acid conditions. It may also be compared with an acid whey product produced by microfiltration, including 15-20% GMP, which was also not acid heat stable.

Thus, the acid heat stable whey protein product of the invention, produced by anion exchange has functional properties and

characteristics which distinguish it from other whey proteins produced from sweet whey, such as by cation exchange methods, particularly with regard to acid heat stability.

The whey protein product of the invention has excellent acid heat stability and is ideally suited for protein fortification of low pH beverages (such as substantially pH 3.2-4.0) such as those marketed for sports nutrition and refreshment. However, the acid heat stable product may also have application for other liquid, solid or semi-solid nutritional or dietary products, such as yoghurts.

Where in the foregoing description, reference has been made to specific components or integers of the invention having known equivalents then such equivalents are herein incorporated as if individually set forth.

Although this invention has been described by way of example and with reference to possible embodiments thereof, it is to be understood that modifications or improvements may be made thereto without departing from the scope or spirit of the invention.

CLAIMS

1. A whey protein product derived from sweet whey by ion exchange using anion exchange medium which is acid heat stable.

2. A whey protein product according to claim 1 which is glyco-macro-peptide (GMP)-delpete.

3. A whey protein product according to claim 2 wherein the total protein content includes no more than 15% GMP.

4. A whey protein product according to claim 3 wherein the total protein content includes no more than 10% GMP.

5. A whey protein product according to claim 4 wherein the total protein content includes no more than 3% GMP.

6. A whey protein product according to any one of claims 1-5 wherein the acid heat stability is such that at pH 3.6 the application of heat does not result in any observable lack of clarity.

7. A whey protein product according to any one of claims 1-5 wherein the acid heat stability is such that the absorbance at 610nm at a pH of substantially 3.6 and heating to a temperature of substantially 80 °C for substantially 20 minutes, of a solution containing substantially 5% protein, is $\leq$ 0.03 AU/cm.

8. A whey protein product according to claim 7 wherein the absorbance is $\leq$ 0.01 AU/cm.

9. A method of producing a whey protein product which is acid heat stable from sweet whey, the method including the steps of:
   - taking a sweet whey stream;
   - acidifying the stream to a pH in the range substantially 4 to 6;
   - subjecting the acidified stream to ion exchange with anion exchange medium; and
   - collecting the non-bound protein stream.

10. A method according to claim 9 further including microfiltration of the sweet whey stream.

11. A method according to either claim 9 or claim 10 further including ultrafiltration of the non-bound protein stream.

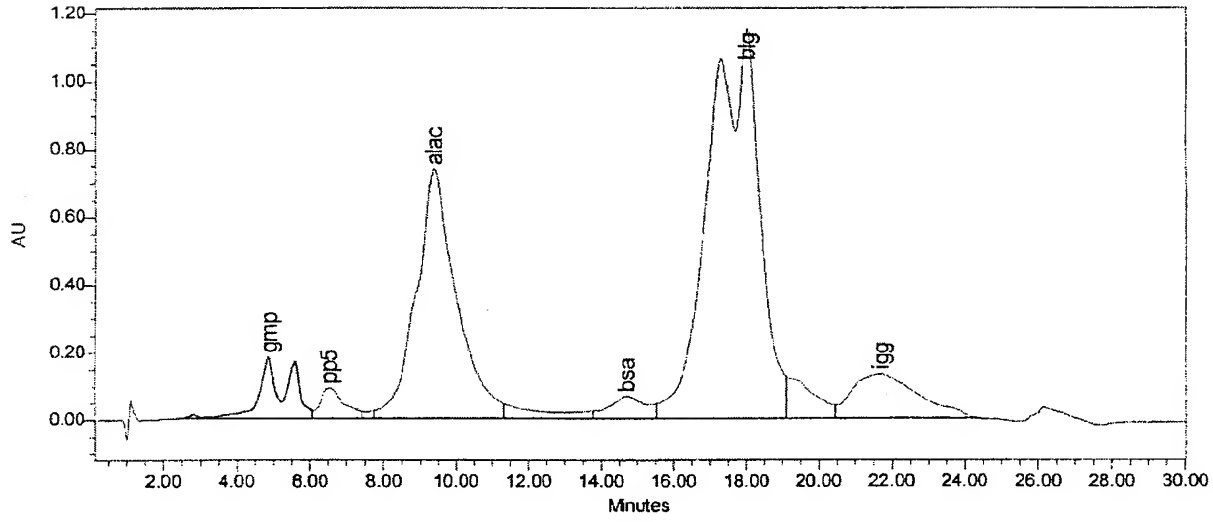
12. A method according to claim 11 wherein the non-bound protein stream is ultrafiltered and diafiltered with water to produce a retentate having a total solids content of 18-28% total solids with a protein content of substantially 90% or greater on a dry basis.

13. A low pH beverage including a whey protein product of any one of claims 1-8.

14. A nutritional product including a whey protein product of any one of claims 1-8.

15. A whey protein product substantially as herein described and with reference to the accompanying figures and/or examples.

16. A method of producing a whey protein product, the method substantially as herein described and with reference to the accompanying examples.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26.00
28.00
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Minutes

FIGURE 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ00/00246

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A23C 21/00, A23C 9/146

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See "electronic data base" box below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPIDS: ion()exchange?, anion()exchange?, A23C-021/IC;
CA, FSTA: whey, anion()exchange?, protein?, ?peptide?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E,X	US 6 168 823 (ETZEL) 2 January 2001 See column 4, paragraph 2; claims	1-16
X	US 5 968 586 (ETZEL) 19 October 1999 See column 7, paragraph 9; claims	1-16
X	WO 95/19714 (VALIO OY) 27 July 1995 See claims	1-16

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 April 2001	24 April 2001
Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	**JAMIE TURNER** Telephone No : (02) 6283 2071

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ00/00246

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97/26797 (AYERS et al.) 31 July 1997 See table 1; page 8, paragraph 4;claims	1-16
X	WO 99/18808 (WISCONSIN ALUMNI RESEARCH FOUNDATION) 22 April 1999 See page 16, paragraph 7; claims	1,9
X	Xu et al., *Process Biochemistry* 36 (2000) 393-399 See page 397, paragraph 2	1,9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/NZ00/00246

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	6168823	AU	10735/99	US	5968586	WO	9918808
WO	9519714	AU	14193/95	EP	757522	FI	940316
WO	9726797	AU	14580/97	CA	2242933	EP	876106
		NZ	326490				
WO	9918088	AU	97468/98	BR	9812856	DE	19744400
		EP	1030848				

END OF ANNEX